

What is claimed is:

1. A method for determining the clonality of a T-cell receptor (TCR) rearrangement in a sample, comprising:

extracting nucleic acid from said sample;

5 amplifying said nucleic acid by polymerase chain reaction (PCR) with two or more TCR-specific primers to provide one or more TCR DNA fragments; and

analyzing said TCR DNA fragments using an electrophoretic gel by temporal temperature gradient gel electrophoresis (TTGE), wherein the presence of one or more discrete DNA bands in said electrophoretic gel indicates the presence of a clonal TCR rearrangement.

10 2. The method of claim 1, wherein one or more control DNA samples are analyzed in said electrophoretic gel, and the discrete DNA band(s) in said electrophoretic gel indicating the presence of a clonal TCR rearrangement migrate at a different rate from any discrete bands present in said control DNA sample(s).

15 3. The method of claim 1, wherein said sample is obtained from a human.

4. The method of claim 1, wherein said sample is a blood sample, a lymphatic tissue sample, a paraffin-embedded tissue sample, or a skin biopsy sample.

5. The method of claim 4, wherein said sample is a blood sample, and said nucleic acid is extracted from white blood cells in said blood sample.

20 6. The method of claim 4, wherein said sample is a lymphatic tissue sample, and said nucleic acid is extracted from formalin-fixed lymph node tissues.

7. The method of claim 4, wherein said sample is a skin biopsy sample, and said nucleic acid is extracted from formalin-fixed skin biopsy sample.

8. The method of claim 1, wherein said TCR-specific primers are the DNA sequences set forth in SEQ ID NO:3 and SEQ ID NO:4.

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9. The method of claim 1, wherein said TCR DNA fragments comprise a VJ region of a TCR- γ gene.

10. The method of claim 1, wherein said TTGE comprises the step of uniformly increasing the temperature of said electrophoretic gel.

5 11. The method of claim 10, wherein the temperature of said electrophoretic gel is raised from about 60°C to about 66°C at a gradient rate of 1°C/hour.

12. The method of claim 11, wherein said electrophoretic gel is a polyacrylamide gel containing urea and formamide that is run for 6 hours at 90 volts.

13. The method of claim 1, wherein one or more DNA migration markers are used as a reference to reflect a relative mobility of TCR DNA fragments.

14. The method of claim 13, wherein said DNA migration marker is prepared by cloning and isolating plasmid DNA from one or more DNA samples positive for clonal T-cell rearrangement.

15. A method for diagnosing a patient suspected of having a neoplastic T-cell disease, comprising:

obtaining a sample from said patient; and

determining whether a clonal TCR gene rearrangement is present in said sample by analyzing the sample by the method of claim 1, wherein the presence of said clonal TCR gene rearrangement is indicative of said neoplastic T-cell disease.

20 16. A method for comparing two or more lesions in a patient having a neoplastic T-cell disease, comprising:

obtaining a sample from a first lesion and a second lesion in said patient;

determining whether identical clonal TCR gene rearrangements are present in each of said samples by analyzing each of said samples by the method of claim 1 and comparing the results obtained from said analyses.

17. The method of claim 16, wherein said second lesion is a recurrence at the site of said first lesion.

18. The method of claim 16, wherein said first lesion and said second lesion are from different disease foci in said patient.

5 19. A method of preparing a DNA migration marker for use in a TTGE gel, comprising:
providing one or more TCR DNA segments having known migration rates in said TTGE gel.

20. The method of claim 19, wherein said providing step comprises:

(a) amplifying one or more DNA samples comprising clonal TCR rearrangements using
10 TCR-specific primers to provide one or more clonal DNA fragments;

(b) inserting each said clonal DNA fragment(s) in one or more plasmids;

(c) isolating each said plasmid(s); and

(d) amplifying the clonal DNA fragment in each said plasmid(s) to provide said one or more
15 TCR DNA segments.

21. A TTGE DNA migration marker, comprising:

a substantially pure DNA molecule comprising a TCR gene sequence; and

a buffer suitable for loading on a TTGE gel.